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A Genetic Analysis of the Common Squid, *Todarodes pacificus* in the Korean Waters

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ABSTRACT

In order to estimate the genetic variability and differentiation in common squid, eleven isozymic loci, coded for nine enzymes detected by starch gel electrophoresis, were scored from nine spawning cohorts in four localities.

The expected average heterozygosity ranged from 0.00019 (between II-S_2 and Na-W) to 0.00814 (Between Bu-S and Na-W) in nine different spawning cohorts in four localities.

A dendrogram, based on genetic distance mentioned, illustrated that nine different spawning cohorts were divided into three groups, similar to the result estimated by their ecological characterizations. From these results, we estimate that the common squid distributed throughout Korean waters will maintain this gene exchange. It is postulated that either the summer or the autumn spawning cohort has developed a local population that is isolated by hydrographic factors.

Keywords: *Todarodes pacificus*, Electrophoresis, Enzyme, Genetic distance, Hydrographic factors.

INTRODUCTION

Todarodes pacificus belongs to the family Ommastrephidae. The spawning grounds and migration pattern of this species includes the waters of Korea, Japan, and North Pacific ocean (Nasu *et al.*, 1991)

Summer, autumn and winter spawning cohorts are seperated in mantle length distributions on the basis of accumulated size and maturation by Tanaka's method (Akamine, 1982).

With the exception of the summer spawning cohort, each spawning cohort is sexually homogeneous. The summer spawning cohort shows significants sex variation in term of relative growth between mantle length and body weight. Three spawning cohorts show significant differences in morphological terms (Kim *et al.*, 1997).

These three cohorts were easily distinguishable in terms of spawning period and fecundity, and they were slightly different in frequency distribution of egg size. They were not significantly different in mantle length at 50% maturity (Kim *et al.*, 1997).

The purpose of this study is to examine the genetic polymorphism of each cohort. We will use the genetic method, and we will compare our results with, Kim *et al.*'s morphological and ecological research.

The isozyme analysis method, the mitochondria heredity analysis and the Random Amplified Polymorphic DNA Assay (RAPD) have been used to analyse genetic variability and differentiation within a specific species. In the isozyme analysis, the genetic differences within a species depends on the variations in genetic frequency, and diversity between species is expressed in the difference of alleles. Therefore, we use this analysis to investigate the genetic differences.

The reaction of a squid cohort to isozyme was

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Table 1. Sampling date, localities and number of individuals of common squid, *Todarodes pacificus*

Date	Number of individuals	Localities	Spawning cohorts	Abbreviation of samples	Mantle length* (cm)	Body weight* (g)
7 June	56	Busan	Autumn	Bu-A	14.71 ± 1.27	61.67 ± 15.77
10 June	50	Busan	Summer	Bu-S	20.00 ± 3.12	179.08 ± 44.89
12 June	52	Ilgwang	Summer	Il-S1	21.39 ± 1.25	193.96 ± 31.87
12 June	62	Namhae	Winter	Na-W	10.50 ± 0.86	24.59 ± 6.07
24 June	49	Ilgwang	Summer	Il-S_2	19.88 ± 1.83	177.63 ± 41.92
1 July	53	Pohang	Autumn	Po-A_1	16.63 ± 1.31	95.32 ± 20.37
3 July	62	Pohang	Autumn	Po-A_2	15.89 ± 1.08	84.82 ± 18.99
4 July	51	Pohang	Winter	Po-W	13.19 ± 2.16	46.62 ± 13.40
20 July	47	Pohang	Summer	Po-S	23.20 ± 1.54	293.10 ± 65.61

investigated by Natsukari (1989), who studied the activation of enzymes in muscle and liver tissue, and Fujio (1984), who studied the oral bulb muscles in six species of a squid cohort, then studied the genetic variation and relationship according to the collection locations.

This study will be to investigate the activation of enzymes and the relationship between alleles, to assess the extent of variability, and to analyze the population structure of *Todarodes pacificus*.

MATERIALS AND METHODS

The samples consisted of the nine different spawning cohorts in four localities of Korean waters from June, 1994 to May, 1995. Sampling localities and general data are shown in Fig. 1 and Table 1. The spawning cohort used a defined group; every squid was born at the exact same time.

The starch gel electrophoresis was carried out according to standard procedures (Shaklee *et al.*, 1990). A total of eleven enzymes were surveyed, namely: Aspartate aminotransferase (AAT), acid phosphatase (ACP), creatine kinase (CK), diaphorse (DIA), esterase (EST), α-glycerophosphate dehydrogenase (αGPD), glucose-6-phosphate isomerase (GPI), isocitrate dehydrogenerase (IDH), leucine aminopeptidase (LAP), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), malic enzyme (ME), manno-sephosphate isomerase (MPI), 6-phosphogluconate dehydrohenase (G6PD), phosphoglucomutase (PGM), sorbitol dehydrogenase

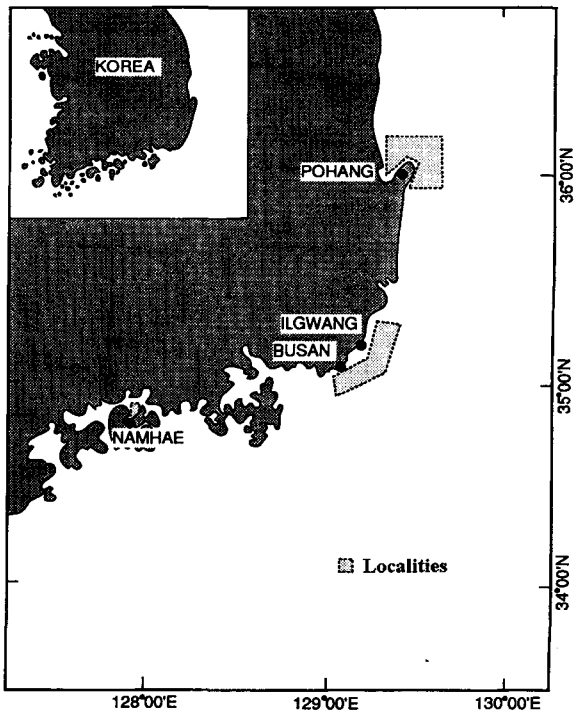

Fig. 1. Map showing the fishing localities of common squid, *Todarodes pacificus*.

Table 2. Detected isozyme and allele frequency at the 15 gene loci based on 11 enzymes in the nine different spawning cohorts in the four localities of the common squid, *Todarodes pacificus*

Enzyme	Locus	Allele	Bu-S	Il-S_1	Il-S_2	Po-S	Bu-A	Po-A_1	Po-A_2	Na-W	Po-W
		n	50	52	52	47	57	53	62	62	51
AAT	Aat-1	a	0.000	0.010	0.000	0.000	0.045	0.028	0.016	0.008	0.000
		b	1.000	0.990	1.000	1.000	0.955	0.972	0.976	0.992	0.990
		c	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.010
	Aat-2	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	-	-
DIA	Dia	a	0.023	0.010	0.011	0.000	0.000	0.000	0.000	0.000	0.000
		b	0.757	0.692	0.712	0.776	0.759	0.875	0.713	0.686	0.730
		c	0.174	0.250	0.200	0.181	0.205	0.083	0.221	0.218	0.218
		d	0.023	0.019	0.033	0.032	0.018	0.042	0.041	0.056	0.013
		e	0.023	0.029	0.033	0.011	0.009	0.000	0.025	0.024	0.013
		f	0.000	0.000	0.011	0.000	0.009	0.000	0.000	0.000	0.000
αGPD	αGpd	a	1.000	0.990	1.000	1.000	1.000	1.000	1.000	0.992	1.000
		b	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.008	0.000
GPI	Gpi	a	1.000	1.000	1.000	1.000	1.000	-	1.000	1.000	1.000
IDH	Idh	a	0.000	0.019	0.000	0.000	0.009	0.000	0.008	0.008	0.020
		b	0.020	0.000	0.000	0.043	0.000	0.019	0.016	0.000	0.049
		c	0.980	0.971	0.990	0.946	0.964	0.981	0.944	0.9562	0.891
		d	0.000	0.010	0.010	0.011	0.018	0.000	0.024	0.016	0.020
		e	0.000	0.000	0.000	0.000	0.009	0.000	0.008	0.024	0.020
MDH	Mdh-1	a	0.000	0.010	0.082	0.000	0.000	0.000	0.008	0.000	0.010
		b	1.000	0.990	0.918	1.000	1.000	1.000	0.992	1.000	0.990
MPI	Mpi-1	a	0.452	-	0.471	0.414	0.557	-	0.400	-	0.233
		b	0.548	-	0.529	0.586	0.443	-	0.600	-	0.767
	Mpi-2	a	0.631	-	0.786	0.871	0.843	-	0.900	-	0.800
		b	0.369	-	0.214	0.129	0.157	-	0.100	-	0.200
ME	Me-1	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	Me-2	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
G6PD	G6pd	a	0.020	0.000	0.020	0.011	0.018	0.000	0.008	0.024	0.000
		b	0.920	0.827	0.848	0.808	0.812	0.849	0.807	0.855	0.882
		c	0.020	0.029	0.010	0.043	0.054	0.009	0.040	0.008	0.049
		d	0.040	0.144	0.122	0.138	0.098	0.142	0.145	0.113	0.069
		e	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000
PGM	Pgm	a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000
		b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.995	1.000
SOD	Sod	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
P^*			0.182	0.182	0.273	0.273	0.182	0.182	0.273	0.182	0.273
P			0.091	0.364	0.091	0.091	0.182	0.182	0.182	0.273	0.182
$P + P^*$			0.273	0.546	0.364	0.364	0.364	0.364	0.455	0.455	0.455
He			0.053	0.038	0.081	0.074	0.079	0.052	0.085	0.078	0.080

P^*: Proportion of polymorphic loci whose most common allele is not greater than 0.95 in frequency.
P: Proportion of polymorphic loci whose most common allele is greater than 0.95 in frequency.
He: Expected mean heterozygosity.
n: Number of individuals.
Abbreviations of the samples are listed in Table 1.

(SDH), and superoxide dismutase (SOD).

Electrophoretic techniques, starting procedures, genetic interpretation of zymogram patterns and locus designations followed those of Fujio (1989), Kijima and Ochiai (1988) and Shaklee *et al.* (1990). The genetic distance was calculated using the formula proposed by Nei (1972), the dendrogram was constructed by UPGMA.

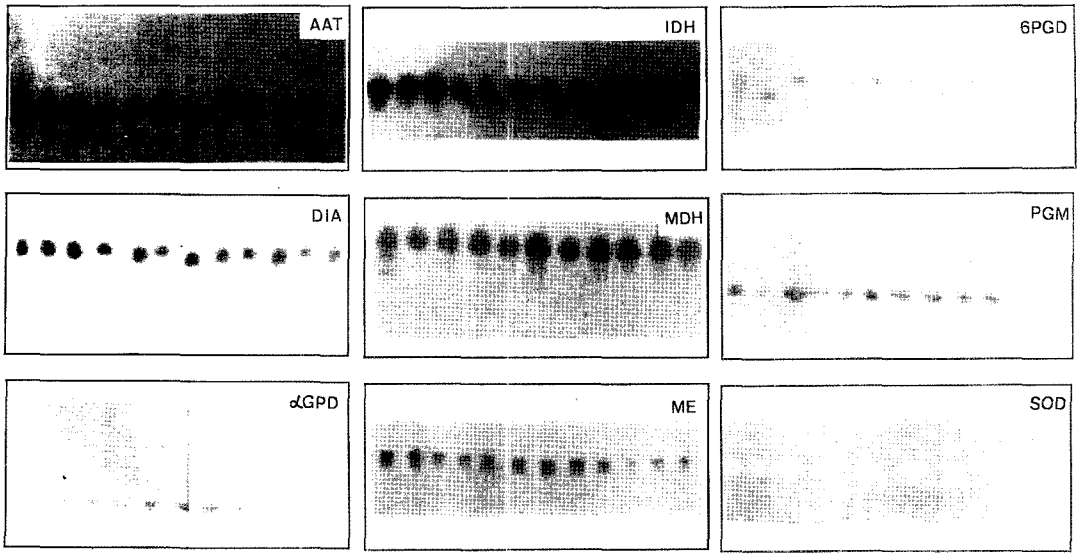

Fig. 2. Electrophoretic band patterns of nine enzymes in common squid, *Todarodes pacificus*.

RESULTS

1. Genetic control of isozymes

Among the 17 enzymes examined, nine enzymes (AAT, DIA, αGPD, IDH, ME, MDH, G6PD, PGM and SOD) were detected in all of the spawning cohorts. The electrophoretical patterns of isozymes are shown in Fig. 2.

Single zones appeared in six enzymes, namely DIA, αGPD, IDH, G6PD, PGM, SOD. Therefore, we estimate that each of these enzymes is controlled by at least one locus. Two seperate zones appeared in three enzymes (AAT, MDH, ME). From these patterns of double independent bands, two loci were assumed to control them.

Table 3. Estimates of t-test between every possible paris (below diagonal) and Nei's genetic distance (upper diagonal) of the nine different spawning cohorts in the four localities of the common squid, *Todarodes pacificus* based on allele frequency at 11 gene loci.

Samples*	Bu-S	Il-S_1	Il-S_2	Po-S	Bu-A	Po-A_1	Po-A_2	Na-W	Po-W
Bu-S	-	0.00144	0.00073	0.00134	0.00105	0.00203	0.00138	0.00814	0.00096
Il-S_1	2	-	0.00029	0.00086	0.00068	0.00311	0.00027	0.00035	0.00108
Il-S_2	3	2	-	0.00061	0.00062	0.00214	0.00032	0.00019	0.00090
Po-S	2	0	2	-	0.00045	0.00115	0.00034	0.00082	0.000101
Bu-A	3	0	4	0	-	0.00168	0.00030	0.00069	0.00098
Po-A_1	1	1	5	0	2	-	0.00212	0.00254	0.00277
Po-A_2	2	0	2	0	0	2	-	0.00035	0.00082
Na-W	1	0	2	0	1	2	0	-	0.00075
Po-W	2	2	4	0	3	3	0	1	-

*Abbreviations are listed in Table 1.

2. Genetic Variation and Differentiation of Nine Different Spawning cohorts

The obtained allele frequency for nine different spawning cohorts are shown in Table 2.

The degree of genetic variation of the nine different spawning cohorts in four localities was examined with 11 isozyme loci. The range of the average number of alleles per locus was 1.64-2.18. The range of the polymorphic rate with a greater than 0.95 degree of genes was 0.091-0.364, the rate with less than 0.95 was 0.182 and 0.273, respectively. Thus, the range of the polymorphic rate, which shows the group variation, was from 0.273 to 0.546 and the average range of He was from 0.038 to 0.085.

To find out the level of genetic variability among these cohorts, the difference in genetic frequencies was analyzed using a t-test as shown in Table 3. At least 24 combined sections among the 36 sections in the nine cohorts were different in more than one locus. Therefore, it was found that the nine cohorts formed an independent group structure.

As according to Nei's genetic distance the samples were examined based on their genetic frequency (Table 3). Note that the genetic distance between Il-S2 in the summer spawning cohorts and Na-W in the winter spawning cohorts is the shortest, yet that between Il-S1 in the summer spawning cohorts and Po-A1 in one of the autumn spawning cohorts is the longest. Thus, the genetic distance ranged from 0.00019 to 0.00311.

To find out the genetic relationship among the nine different spawning cohorts in four localities, the genetic distance was showed in a dendrogram (Fig. 3). The genetic distance between Il-S_2 in the summer spawning cohort and Na-W in the winter spawning cohort was the shortest at 0.00019 and that between Il-S1 in the summer spawning cohort and Po-A_2 in the autumn spawning cohort was the second shortest at 0.00027. The genetic distance between the two groups, Il-S_2-Na-W and Il-S_1-Po-A_2 was 0.00033. The genetic distance between Po-S in the summer spawning cohort and Bu-A in the autumn spawning cohort was 0.00045 and their distance from the previous group was 0.00062. The distance of Po-W in the winter spawning cohort within the previous group was 0.00094 and it is involved in the same group. The distance between

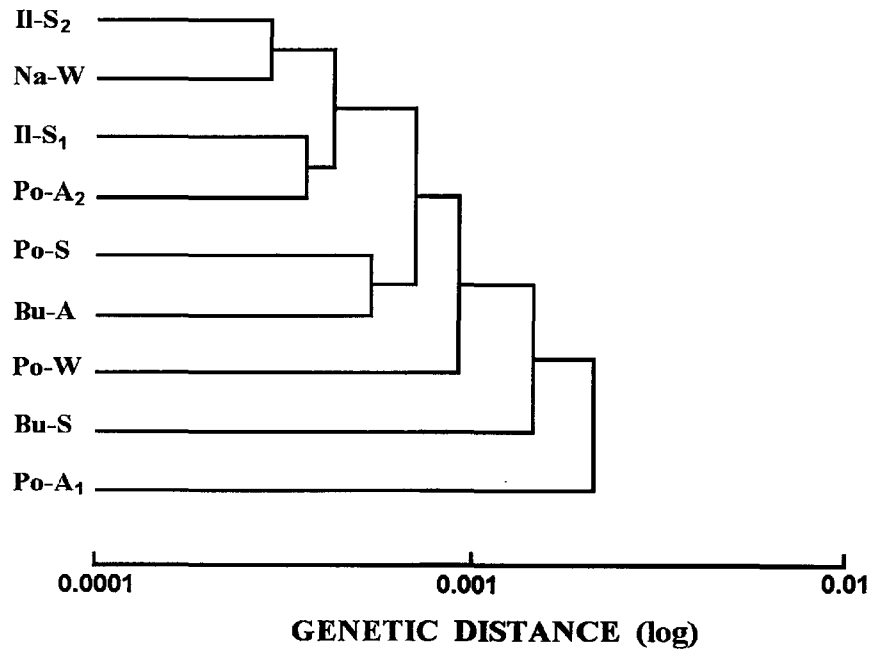

Fig. 3. Genetic relationship among nine sample lots of common squid, *Todarodes pacificus*.

Bu-S in the summer spawning cohort and Po-A_1 in the autumn spawning cohort was larger than 0.001. Therefore, the nine cohorts can be divided into three groups at a 0.001 level of genetic distance.

From these results, we estimated that the summer (Il-S_1, Il-S_2, Po-S), autumn (Bu-A, Po-A_2) and winter (Na-W, Po-W) spawning cohorts were populations with changing genes caused by spawning ecology, oceanic current, and so on.

It was, however, found that Bu-S in the summer spawning cohort and Po-A1 in the autumn spawning cohort were separated and divided into independent spawning cohorts in different localities.

DISCUSSION

According to Nei (1972), if the average genetic distance based on the isozyme genetic frequency was about 1.0, it was considered to be a species, if 0.1, it was considered to be a sub-species, if 0.01, it was considered to be a local species, and if it was about 0.001, it was a member of a inter-species.

In fish species, Fujio (1989) reported an average genetic distance among species as 0.3888, among populations as 1.392. The present study is complete agreement with the above reports.

The genetic differentiation of the species was also examined; the level of the overall genetic differentiation was very low and the creatures were found to be within the range of the species. However, when the apexes were assumed to be in the same species, the relationship was found to be distant though those apexes; the relationship was closer when the apexes were assumed to be in different species. As a result, it was found that the expected species did not necessarily agree with the genetic structure.

The differences of the genetic frequencies were discovered dividing by forming the nine different spawning cohorts of four localities into two groups and it was found that the differentiation level of cross spawning was low. This was true despite low genetic differentiation. It was found that the cohorts of squid were cross spawning and the genes were exchanged in the spawning cohort even though they migrated in the spawning cohort. Bu-S in the summer spawning cohort and Po-A_1 in the autumn spawning cohort formed an independent groups that hardly mixed with the other seven groups, as they were separated from oceanic currents and the geographical environment.

The genetic variability and differentiation of a larger number of samples from different locations should be investigated and the geographical factors, environmental factors, migration processes should e compared. In further mitochondria and a RAPD genetic analyses the mtDNA and the DNA from squid populations will be extracted..

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